were incubated at 37˚C for 4 hours. A reading of each well was done using the MAXline Microplate Readers to evaluate pemetrexed IC50 value. Microarray analysis was performed with Affymetrix U-133 A&B and plus2 chips to detect putative biomarker genes which predict pemetrexed sensitivity. Data analysis with obtained chip data was made with GeneSpring software. The relative fluorescent intensity by microarray was validated by RT-PCR on the putative gene, Synovial Sarcoma X3 (SSX3).

Results: Three NSCLC cell lines (H157, A549 and H1648) had over 100 nM/L IC50 value (high IC50), and remainder NSCLC cell lines (H2122, H1975 and H358) had under 20 nM/L (low IC50). In GeneSpring analysis, 12 genes which were 5 fold higher in sensitive cell lines than in resistant cell lines were chosen. As SCLC cell lines are usually floating and difficult to select resistant cell lines, 3 cell lines were selected as sensitive lines with low IC50 values (18-34 nM/L). Then 3 genes out of 12 genes were selected which were compatible in 3 sensitive SCLC cell lines. They were Slit 2, SSX3 and EST. Sense and antisense primers were made for RT-PCR, and SSX3 microarray results were validated with those.

Conclusion: These results need to be verified in samples from clinical cohorts, which are NSCLC, SCLC and malignant pleural mesothelioma. It was suggested that SSX3 would predict the pemetrexed sensitivity and extend the indications of that treatment.

P2-048 **BSTB: Molecular Targets Posters, Tue, Sept 4**

Activity of epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) in chemotherapy-pretreated non-small cell lung cancer (NSCLC) patients (pts) prospectively selected according to specific molecular predictive factors

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Background: EGFR TKIs are effective in a small proportion of chemotherapy-pretreated NSCLC pts. A number of molecular alterations along the EGFR pathway have been proposed as predictors of response to EGFR TKIs, but to date none of them has been prospectively validated.

Methods: Advanced, chemotherapy-pretreated, NSCLC pts were screened for the following EGFR pathway alterations: EGFR gene mutations (by SSCP and sequencing) and/or increased copy number (by FISH), EGFR protein expression (by IHC), and HER-2, phosphorylated AKT (pAKT), and total AKT protein expression (by IHC). Upon progression, pts were then assigned to treatment with an EGFR TKI (Iressa™ or Tarceva™) according to one of the following groups: A) mutated (regardless of any other parameter); B) non-mutated, amplified (either true gene amplification or high-grade polysomy in > 40% of the tumor cells); C) non-mutated, non-amplified, EGFR positive (IHC score ≥ 1+); D) Adk or BAC histology and no smoking history (non-mutated, non-amplified, EGFR-negative pts or unknown EGFR status).

Results: From January 2005, 129 pts were screened for EGFR pathway alterations, while undergoing chemotherapy. Thirteen pts were identified in group A (6 with exon 19 deletions, 6 with exon 21 point mutations, 1 with both); 39 pts in group B (including 7 pts with true gene amplification); 32 pts in group C (including 19 pts with a pAKT IHC score ≥ 1+); 7 pts in group D (including 2 pts with BAC histology).

Treatment results in the different subgroups are reported in Table 1.

	A	B	C	D
N. treated	11	25	25	5
PR	2	2	1	1
SD ≥ 6 mos	2	5	4	0
SD < 6 mos	2	5	4	2
PD	4	9	15	1
Too Early	1	4	1	1
Awaiting treatment	2	14	7	2

Conclusions: Based on the data available so far, there seems to be a continuum of response/ disease control rate that progressively declines from group A to group C. Accrual to the study will be continued in all 4 subgroups until computer simulations using a continual reassessment method will allow to confidently exclude a true response rate ≥ 10% in any of the groups.

P2-049 **BSTB: Molecular Targets Posters, Tue, Sept 4**

Cytochrome P450 expression in non-small cell lung cancer

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Background: The cytochrome P450 (CYP) is associated with tumor development and progression as well as activation of anti-cancer prodrugs and their metabolic clearance. Recently, some CYPs, in particular enzymes of the CYP3A subfamily (CYP3A4, CYP3A5, and CYP3A7), have been found to play a role in the metabolism of many anticancer drugs. CYP3A not only inactivates major anticancer drugs, such as tamoxifen, taxol and vinca alkaloids, but also activates major anticancer prodrugs, such as cyclophosphamide and ifosphamide. For the better management of lung cancer, it is essential to understand the roles of CYPs and the relationships between their expression and the clinical feature of individual lung cancers. In this study, we investigate the expression of four CYPs (CYP1A1, CYP2A6, CYP2E1 and CYP3A) in 78 non-small cell lung cancer (NSCLC) and their relationships to each other, tumor p53 expression, and clinical features of the patients.

Materials and Methods: We examined CYPs and p53 immunoreactivities in 78 Japanese patients with NSCLC who underwent surgical resection at UOEH after obtaining appropriate informed consent. Histological typing of the tumors was performed according to the WHO classification (adenocarcinoma; 48 cases, squamous cell carcinoma; 30 cases). Immunohistochemical staining (IHC) of 78 NSCLC was performed by the LSAB (labelled streptavidine biotin) method. CYPs and p53 expression was determined as positive when more than 10% of tumor cells were stained. The p53 mutations in 48 adenocarcinomas were sequenced by the dideoxy chain termination method using an ABI 373A DNA Sequencer (Applied Biosystems).

Results: The CYP1A1, CYP2A6, CYP2E1 and CYP3A positive rates in 48 adenocarcinomas were 43.8% (21/48), 45.8% (22/48), 39.6%